REMARKS

Claim 1 has been amended. Support for the amendment can be found in Examples 3, 4, 7-15 and in paragraph [0044] of the Specification as filed. Therefore, no new matter has been introduced by this amendment. Claim 43 has been canceled without prejudice. The following addresses the substance of the Office Action.

Non-obviousness

The Examiner has rejected Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161). More specifically, the Examiner alleges that it would have been obvious to one skilled in the art at the time the invention was made to modify the method of Anthony by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

> To establish a *prima facie* case of obviousness, the PTO must cite one or more references that provide some suggestion or motivation to modify the references to achieve the claimed invention, provide a reasonable expectation of success to achieve the claimed invention, and finally, the cited art must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). Here, the cited art either taken alone or in combination, fails to provide any of the required factors.

The present invention is related to a method of using arrays comprising covalently bound capture nucleotide sequences wherein these sequences comprise a spacer and a sequence that specifically binds to the target; two categories of capture nucleotides sequences for group and sub-group detection and primer pairs capable of amplifying at least two of 4 homologous sequences. In Claim 1, these single-stranded capture nucleotides sequences are covalently bound to the solid support and include a spacer that places the specific sequence of the capture nucleotide sequence such that it is able to hybridize with the corresponding target nucleotide sequence at a certain distance from the solid support surface (at least 40 bases). The binding between the target nucleotide sequence and its corresponding capture nucleotide sequence forms a signal at the expected location (the location of the specific capture nucleotide sequence), the detection of said signal allowing a discrimination of a target sequence from other homologous sequences obtained from other organisms.

Furthermore, in contrast to the presently claimed method in which the capture nucleotide sequences are covalently bound to an insoluble support by a spacer, Anthony et al. teaches the use of short capture probes of 20-25 bases which do not include a spacer and are immobilized on nylon membranes. The binding of the capture probes on filter or membrane means that there is no control of which part of the sequence would be available for hybridization (Anthony et al. pg. 783, line 2: "The length of the UV exposure used to link the probe on the nylon was found to have a marked effect on the intensity of the resulting spots"). Therefore, because there is no single point attachment of capture probes on nylon membranes, the addition of a spacer to the capture probes is not compatible with the method of Anthony et al. In contrast to the method of Anthony, the utilization of spacers in the present method ensures that the portions of the capture nucleotide sequences which are complementary to the target sequences are available for hybridization. Therefore, Anthony in fact teaches away from the present invention.

Additionally, in the method of Anthony et al. one target sequence can cross-react with several capture probes (note that some of the filters depicted in Fig. 1 of the Anthony reference contain more than one position of hybridization). In such cases, it is the pattern of several positive spots which allows specific identification of the organism present. This means that the interpretation of the result is not straightforward. Contrary to method which utilize a pattern of spots (i.e. Anthony et al.), in the embodiment of Claim 45 a single "spot signal" directly allows the identification of a specific organism, therefore one capture nucleotide sequence is sufficient for the identification of one target nucleotide sequence thus permitting correlation between intensity of the spot signal and the amount of target nucleotide sequence present.

Combination of references

Shchepinov et al. describes the use of a chemical spacer to reduce steric interference of the support on the hybridization behavior of immobilized oligonucleotides. The support used by Shchepinov et al. is polypropylene which is highly hydrophobic despite amination (see page 1158, left column). If the oligonucleotide is fixed directly onto the surface of a hydrophobic support, there would be strong repulsion during hybridization with a complementary strand. The authors state that negatively charged group in the spacer diminishes the yield of hybridization, and describe the preferred use of a spacer with low negative charge density therefore by stating that high negative charge in the spacer could act to repel the target and hence reduce the rate of

duplex formation are "teaching away" from the invention in which the spacer is a negatively charged nucleic acid sequence. Furthermore, the authors hybridize with complementary single-stranded short oligonucleotides (12 nucleotides) or with tRNA which is also single-stranded. Additionally, the optimal chemical spacer is 30-60 atoms in length which would be equivalent to 15-30 nucleotides in length (see figure 2). Duplex yield declines with further increase in the length of the spacer. At 30 units (180 atoms or 90 nucleotides), the yield of hybridization is the same as that with no spacer at all (figure 3b).

In the present invention, the inventors have used a spacer made of nucleotides sequences of at least 40 nucleotides which are negatively charged (at least 40 negative charges). The yield of hybridization is still increasing with a spacer of 60 nucleotides (see Figure 3 as filed). The unexpected advantage of the method of the present invention is that a very good yield of hybridization of amplified long target DNA was obtained even in the presence of its complementary nucleotide sequence present in solution. The hybridization is performed with double-stranded amplified by PCR and present in solution target DNA. The dsDNA reassociate much faster in solution than to hybridize on small sequences fixed on a solid support where diffusion is low, thus reduce even more the rate of the reaction. The difference of the claimed method is the use of small specific sequences on capture probes in order to be able to differentiate between homologous sequences while having a high yield of hybridization on the immobilized probe (see paragraph [0044] of the Specification as filed).

Shchepinov et al. neither mention nor suggest the use of spacer of at least 40 nucleotides. In fact, the optimal spacer length of Shchepinov et al. (equivalent to 15-30 nucleotides) is not within the range of the present invention (above 40 nucleotides). As shown in the figure 3 of the Specification as filed, the rate of hybridization is increased by a factor of ~2 for a spacer of 60 nucleotides as compared to a spacer of 20 nucleotides while there is no increase at this size in the paper of Shchepinov et al.

Additionally, Shchepinov et al. method is not related to a detection of homologous sequences contrary to the present invention. The solution proposed by the present invention is to obtain both a specific and sensitive detection of multiple homologous sequences which are present in solution as amplified double-stranded nucleotide sequences. Hybridization conditions are much more stringent in case of homologous sequences in order to differentiate small

differences in their sequences, which still reduce the hybridization rate. Results are therefore hardly comparable.

The high yield of hybridization is an important aspect of the present invention since it allows the surface bound capture probes to efficiently compete with the reassociation of the target with its complementary sequence present in the same solution. The inventors found that a spacer of at least 40 bases increases the specificity of hybridization of the capture molecule with the target molecule. The spacers of Shchepinov et al. are built up from monomeric units, using phosphoramidite chemistry by condensation onto an amine functionalized polypropylene support. This means that they are synthesized directly on the support according to Southern's methods. Long polynucleotides including a spacer of more than 40 nucleotides and a specific sequence of 15-40 nucleotides cannot be directly synthesized on the support due to limits of the *in situ* synthesis. Indeed, current coupling efficiencies impose a limit of ± 25 bases to these chips. Beyond this limit, the accumulation of incomplete synthesis products becomes a problem. The spacer of the present invention is not synthesized directly on the support but by conventional chemical synthesis. The specific part of the capture probe may be synthesized consecutively to the spacer nucleotide bases resulting in a single molecule. The synthesized capture nucleotide sequences are then covalently fixed on the support according to an array by physical deposition. The single molecules thus obtained highly simplify and reduce the cost of production of the capture nucleotide sequences.

For these reasons, it would not have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Anthony et al. so as to have used a chemical spacer of Shchepinov et al. These references both fail because neither provides the requisite motivation to combine, the reasonable expectation of success, nor teach all the limitations of the claimed invention.

Furthermore, the inventors completed the invention of this application prior to February 2000, the date that appears on Anthony et al. publication, and therefore the cited reference does not constitute prior art. The Declaration under 37 CFR §1.131 supporting this assertion will be submitted shortly.

Therefore, Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 are not obvious over the cited references and their rejection under 35 USC §103(a) should be withdrawn.

The Examiner has rejected Claim 15 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161) as applied to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above and further in view of Vannuffel et al. (WO 99/16780). More specifically, the Examiner alleges that because Vannuffel et al. teaches the detection of the *FemA* gene of *Staphylococci* species, it would have been obvious to combine this teaching with the method of Anthony et al. as modified by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

Vannuffel's disclosure of the use of the *FemA* gene of *Staphylococci* species to detect bacteria present in a sample does not provide motivation to combine arrays comprising covalently bound probes of the lengths recited in the claims which comprise a spacer of at least 40 nucleotides in length, and primer pairs capable of amplifying at least two of 4 homologous sequences in methods for detecting specific nucleotide sequences having a homology level higher than 60% with sequences from other organisms. Furthermore, Vannuffel fails to cure the deficiencies of Anthony et al. combined with Shchepinov et a. as discussed with regards to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above. Thus, Vannuffel *et al.* fails to correct the failure of Anthony *et al.*, and Shchepinov *et al.* to render the claimed invention obvious for the reasons addressed above. Therefore, dependent Claim 15 is also non-obvious.

The Examiner has rejected Claim 18 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161) as applied to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above and further in view of Boon et al. (USP 6,488,932). More specifically, the Examiner alleges that because Boon et al. teach that it is advantageous to detect individual sequences that belong to MAGE family for the diagnosis of tumors, it would have been obvious to combine this teaching with the method of Anthony et al. as modified by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

Boon's disclosure of the use of the MAGE family to diagnose tumors does not provide motivation to combine arrays comprising covalently bound probes of the lengths recited in the claims which comprise a spacer of at least 40 nucleotides in length, and primer pairs capable of amplifying at least two of 4 homologous sequences in methods for detecting specific nucleotide

sequences having a homology level higher than 60% with sequences from other organisms. As discussed above, Anthony et al. and Shchepinov et al. do not disclose all the limitations of the claimed invention, and Boon *et al.* fails to correct the failure of Anthony *et al.*, and Shchepinov *et al.* to render the claimed invention obvious for the reasons addressed above. Therefore, dependent Claim 18 is also non-obvious.

The Examiner has rejected Claims 4, 14 and 19 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161) as applied to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above and further in view of Apple et al. (USP 5,451,512). More specifically, the Examiner alleges that because Apple et al. teach that it is advantageous to detect individual sequences that belong to HLA-A family to help determine potential transplantation donors and teaches the amplified nucleotide sequences for HLA-A detection, it would have been obvious to combine this teaching with the method of Anthony et al. as modified by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

Apple's disclosure of the use of HLA-A family to help determine potential transplantation donors does not provide motivation to combine arrays comprising covalently bound probes of the lengths recited in the claims which comprise a spacer of at least 40 nucleotides in length, and primer pairs capable of amplifying at least two of 4 homologous sequences in methods for detecting specific nucleotide sequences having a homology level higher than 60% with sequences from other organisms. As discussed above, Anthony et al. and Shchepinov et al. do not disclose all the limitations of the claimed invention, and Apple *et al.* fails to correct the failure of Anthony *et al.*, and Shchepinov *et al.* to render the claimed invention obvious for the reasons addressed above. Therefore, dependent Claims 4, 14 and 19 are also non-obvious.

The Examiner has rejected Claims 20 and 22 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161) as applied to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above and further in view of Klein et al. (USP 6,255,059). More specifically, the Examiner alleges that because Klein teaches that it is advantageous to detect sequences that belong to the dopamine of histamine receptors coupled to the G genes family to mediate

transmembrane signaling by external stimuli, endocrine function, carbohydrate metabolism, etc., it would have been obvious to combine this teaching with the method of Anthony et al. as modified by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

Klein's disclosure of the use of sequences that belong to the dopamine of histamine receptors coupled to the G genes family to mediate transmembrane signaling by external stimuli, endocrine function, carbohydrate metabolism, etc. does not provide motivation to combine arrays comprising covalently bound probes of the lengths recited in the claims which comprise a spacer of at least 40 nucleotides in length, and primer pairs capable of amplifying at least two of 4 homologous sequences in methods for detecting specific nucleotide sequences having a homology level higher than 60% with sequences from other organisms. As discussed above, Anthony et al. and Shchepinov et al. do not disclose all the limitations of the claimed invention, and Klein *et al.* fails to correct the failure of Anthony *et al.*, and Shchepinov *et al.* to render the claimed invention obvious for the reasons addressed above. Therefore, dependent Claims 20 and 22 are also non-obvious.

The Examiner has rejected Claim 21 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161) as applied to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above and further in view of Murphy et al. (WO 94/05695). More specifically, the Examiner alleges that because Murphy teaches that it is advantageous to detect sequences that belong to the choline receptors coupled to the G genes family for use in diagnosis of neurological, viral or endocrine pathologies, it would have been obvious to combine this teaching with the method of Anthony et al. as modified by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

Murphy's disclosure of the use of sequences that belong to the choline receptors coupled to the G genes family for use in diagnosis of neurological, viral or endocrine pathologies does not provide motivation to combine arrays comprising covalently bound probes of the lengths recited in the claims which comprise a spacer of at least 40 nucleotides in length, and primer pairs capable of amplifying at least two of 4 homologous sequences in methods for detecting specific nucleotide sequences having a homology level higher than 60% with sequences from other organisms. As discussed above, Anthony et al. and Shchepinov et al. do not disclose all the

limitations of the claimed invention, and Murphy *et al.* fails to correct the failure of Anthony *et al.*, and Shchepinov *et al.* to render the claimed invention obvious for the reasons addressed above. Therefore, dependent Claim 21 is also non-obvious.

The Examiner has rejected Claim 23 under 35 USC §103(a) as being allegedly unpatentable over Anthony et al. (*J. Clin. Microbiol.* 2000 **38**:781-788) in view of Shchepinov et al. (*Nucleic Acids Res.* 1997 **25**:1155-1161) as applied to Claims 1, 2, 9, 10, 13, 13, 16, 17, 38, 40, and 42-45 above and further in view of Waxman et al. (USP 6,207,648). More specifically, the Examiner alleges that because Waxman teaches that it is advantageous to detect sequences that belong to the cytochrome P450 isoforms family for use in treatment of cancer, it would have been obvious to combine this teaching with the method of Anthony et al. as modified by using a spacer of at least 6.8 nm in length as taught by Shchepinov et al.

Waxman's disclosure of the use of sequences that belong to the cytochrome P450 isoforms family for use in treatment of cancer does not provide motivation to combine arrays comprising covalently bound probes of the lengths recited in the claims which comprise a spacer of at least 40 nucleotides in length, and primer pairs capable of amplifying at least two of 4 homologous sequences in methods for detecting specific nucleotide sequences having a homology level higher than 60% with sequences from other organisms. As discussed above, Anthony et al. and Shchepinov et al. do not disclose all the limitations of the claimed invention, and Waxman *et al.* fails to correct the failure of Anthony *et al.*, and Shchepinov *et al.* to render the claimed invention obvious for the reasons addressed above. Therefore, dependent Claim 23 is also non-obvious.

CONCLUSION

In view of the foregoing, Applicants respectfully submit the present application is fully in condition for allowance. If any issues remain that may be addressed by a phone conversation, the Examiner is invited to contact the undersigned at the phone number listed below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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